# On-line MRI sequences for the evaluation of Apple internal quality

Angela Melado-Herreros[a], Maria-Encarnación Fernandez-Valle[b], Natalia Hernandez-Sanchez[a], Tatiana Jimenez-Ariza[a], Bert Verlinden[c], J. Val[d], Pilar Barreiro[a]

[a] *LPF-TAGRALIA, Technical University of Madrid (UPM),ETSI Agrónomos, Avda. Complutense s/n, Madrid, Spain*
[b] *CAI of NMR and electronic spin. Universidad Complutense de Madrid. Av. Juan XXIII, nº 1. Madrid, Spain*
[c] *Flanders Centre of Postharvest Technology (VCBT). Willem de Croylaan 42, 3001 Heverlee, Belgium*
[d] *Estación Experimental de AulaDei (CSIC). Avda. Montañana 1005-50059. Zaragoza. Spain*

**ABSTRACT**

**On-line dynamic MRI requires high-speed sequences with motion correction artifacts. In this study, two alternative motion corrected sequences are proposed and implemented in real-time (FLASH and UFLARE), based on T2* or T2 respectively. Sequence selection is related to the expected contrast effect of the disorder: while watercore enhances bright areas due to higher fluid mobility, internal breakdown potentiates low signal due to texture degradation.**

**Metrological aspects such as repeatability of dynamic images and subsequent histogram feature stability become of major interest for further industrial application. Segregation ability among varyin degrees of disorder is also analyzed.**

## 1 Introduction

Several techniques based on Nuclear Magnetic Resonance (NMR) phenomenon, such as Nuclear Magnectic Resonance Spectroscopy (MRS) and relaxometry (MRR) together with Magnetic Resonance Imaging (MRI), have been explored since the 80's in order to evaluate their applicability to the inspection of internal quality aspects in fruits and vegetables (Hernández-Sánchez et al., 2009). NMR is an especially useful monitoring technique since the signal emitted from a sample is sensitive to the density of certain nuclei, chemical structure, molecular or atomic diffusion coefficients, reaction rates, chemical exchange and other phenomena (McCarthy 1994), which gives an enormous scope for applications. Previous works have shown that MRI is feasible to detect a number of miss quality factors such as freeze injury and seeds in citrus (Hernandez-Sanchez et al., 2004, 2005, 2006; Barreiro et al., 2008).

Most of works in agrofood field have been performed with commercial NMR equipment designed for medical purposes, which are not conceived to deal with the practical constrains that appear for the food industry. Several authors have used stationary MRI for fruit and vegetable detection (Clark et al., 1998; Blasco et al, 2012; Min Kim et al., 2008). Nevertheless, this method is very slow and not suitable for industry implementation, which means that an on-line configuration is necessary in order to decrease the monitoring time and thus, the economical costs. The challenge is the achievement of practical image contrast by working procedures that accomplish the industrial requirements. In this study, several sequences have been studied and adjusted for on-line real time inspection of two different internal disorders in apples: watercore and texture breakdown, while being extensively validated for the first one according to expert and 3D segmentation procedures.

## 2 Material and Methods

### *2.1 MRI equipment and fruit grading line*

MRI experiments were performed on a Bruker BIOSPEC 47/40 (Ettlingen, Germany) spectrometer operating at 200 MHz (4.7 T). All experiments were performed with an actively shielded imaging gradient set and a RF volume coil with an inner diameter of 20 cm.

Fruits were conveyed through the coil at a 30 mm/s rate by means of a specially designed conveyor belt.

### 2.2 Samples

Samples used in this study were apples potentially affected by two different processes: (1) watercore, which is a physiological disorder in which intercellular spaces appear filled with fluid and (2) internal breakdown, which is a disorder due to a improper storage that leads to texture degradation and cavities in the inner tissue.

For watercore, 71 samples of Esperiega cultivar studied during 2012 season were measured both, stationary (20 slices per fruit) and under dynamic conditions (3 replicates without slices selection). For internal breakdown Braeburn cultivar has been studied either under static (20 slices per fruit) and dynamic conditions (1 replicate with slice selection). In this case, as the MR sequence needed to be calibrated, several trials have been performed until the definitive parameters were settled (106 samples tested for sequence calibration). Finally, 12 fruits were imaged with the definitive parameters.

### 2.3 MRI sequences and motion correction

For dynamic acquisition, two alternative motion corrected sequences are proposed (FLASH and UFLARE), based on T2* or T2 respectively. Sequence selection is related to the expected contrast effect of the disorder under study (Fig 1).

A B C

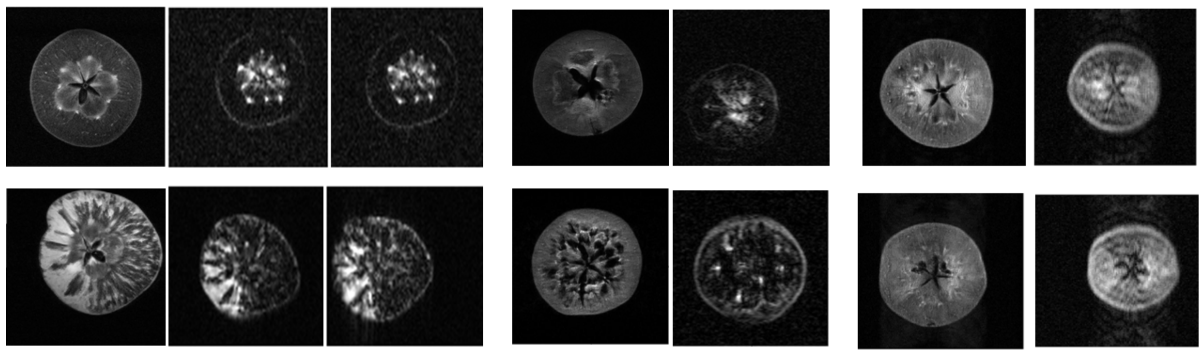

**Fig. 1** Static and dynamic MR images for different disorders. A. On the left part of the image two static MRI with different watercore affection are shown. Images on the right size are two of the three dynamic replicates. B. Images from the left correspond to two static MRI with different internal breakdown affection. On the right part dynamic images acquired with FLASH sequence are shown. C. Images on the left part show static MRI of two different grades of internal breakdown. On the right side the corresponding dynamic images acquired with UFLARE sequence are shown.

In the case of watercore disorder, it enhances bright areas due to higher fluid concentration in the tissues. An eco gradient sequence was selected and hence, the resulting images were T2* weighted. In this type of images, the signal of the healthy tissue has a very low T2* value and then, the signal of the damaged area will be seen brighter than the rest of the tissue, as it presents high T2* values. From all the eco gradient sequences, the one selected was Fast Low Angle Shot sequence (FLASH) as it is the most rapid one and it permitted very low acquisition times, required for on-line inspection. The acquisition parameters were: echo time (TE) 4.5 ms, the acquisition matrix was 128 × 64 and reconstructed to 128 × 128. The field of view (FOV) was 12 × 12. The total acquisition time was 768 ms for each fruit. All images were acquired with no slice selection but a projection of the whole apple was obtained, as watercore development can be in any part of the volume of the apple.

For the internal breakdown disorder, the eco gradient sequences were not successful, as they are T2* weighted and thus, the pulp has very low signal. As internal breakdown in tissues correspond with holes inside the apples, and no signal is detected, it is necessary the use of another kind of sequence, as a spin echo, which is T2 weighted. In this case, the pulp presents a higher T2 value than the damaged areas, so the healthy tissue will be seen brighter than the damaged one. In order to have a rapid sequence, a Fast Spin-Echo (FSE) was selected, specifically a Rapid adquisition with refocused echoes (RARE) sequence, but with a single shot. This sequence is called UFLARE (Ultra-Fast Low-Angle Rapid-Aquisition and Relaxation Enhancement). The acquisition parameters were: echo time (TE) 165 ms, the acquisition matrix was 128 × 128 and the field of view (FOV) was 12 × 12. The total acquisition time was 1475 ms. In this case a slice selection was performed, as internal breakdown usually develops around the core of the apple. Then, the central slice of each fruit was selected.

Image blurring due to phase shift during motion is corrected in the k-space in real time, just after the acquisition of the data and before the image reconstruction.

Also, 2D static images were acquired for both cultivars. These images consisted on tomographies of 20 slices each. A RARE sequence was used and T2-weighed images were obtained with the following parameters: recovery time (TR) 5000 ms, echo time (TE) 60 ms. Field of view 8 × 8 and 3 mm of slice thickness. The acquisition matrix was 256 × 128 and reconstructed to 256 × 256 by zero filling. The total acquisition time was 2 minutes 2 seconds.

In both cases, three experts classified the damage based on visual classification, using the static MR images, into four groups: 1-healthy apple; 2-light damage; 3-medium damage; 4-strong damage.

### *2.4 Image processing and data analysis*

Both static and dynamic images were submitted to a similar analysis; using dedicated procedures based on Matlab 7.0 image processing and PLS toolbox. First of all, automated background segmentation was performed which is straight forward for the **static images** due to their inherent quality (Melado et al., 2012). For the **dynamic images**, two different masks were defined and tested in order to segment the fruit from the background. The first method consisted on the use of a softened logarithmic filter. Then, an automated segmentation level based on Otsu method was defined for each of the images and the largest object was selected. For the second method, first of all a threshold was defined by selecting corner areas of the MRI which always correspond to the background: the image of the fruit always having higher gray level values than those from the background. In this latter case also further Gaussian filter was applied. Then, in both segmentation procedures, a mask was accomplished in order to ensure that the whole fruit was being taken into account in the segmentation while motion artifacts would be minimized. For such purpose, polar coordinates of a circular object with features extracted the main object and represented on the image and thus, a final fruit mask was obtained for removing the background. This procedure was repeated for the three repetitions and then, the histogram of each fruits area of each repetition was obtained..

Validation of the above mentioned segmentation procedure for dynamic images, together with image processing and data analysis was defined on watercored apples (Esperiega) as several additional features from 3D interactive segmentation on static images was available. 3D features included the percentage of damage and the Euler number, computed with the software Avizo Fire 7.0 (Melado et al., 2012. Not published). A scheme of the image processing and data analysis may be seen in Fig. 2.

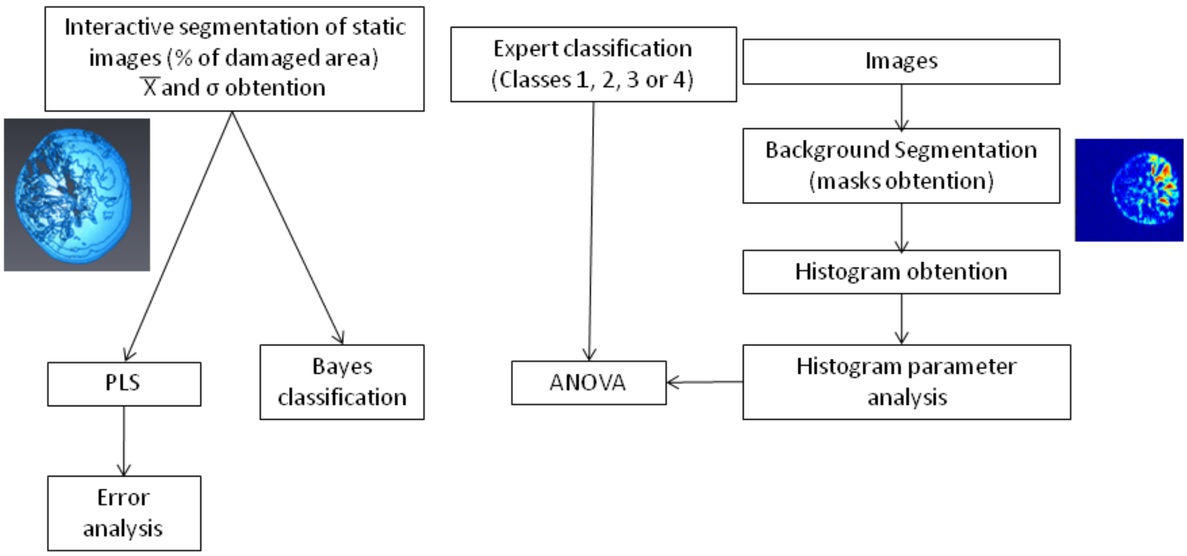


**Fig. 2** Image processing scheme followed.

For both segmentation masks, histogram parameters (mean gray level, variance of the gray level, skewness and kurtosis) were obtained and an ANOVA was carried out regarding damage level and replicate.

Parallel to this analysis, a PLS model for estimating the percentage of damage according to the 3D static image segmentation was carried out for each repetition (Fig 2), using the normalized histograms as independent variables and cross validation with contiguous data blocks; 2 latent variables (LVs) were selected as to minimize the standard error of prediction under cross validation. With the predicted values a regression error analysis was performed, by estimating the error (observed – predicted) and the relative error (error/observed).

Also a Bayesian classification was performed based on the percentage of damage in the static images in order to assess the reliability of expert classification. To this aim the averages and standard deviation of the expert defined classes were used, and each fruit was reassigned to a new class, for which the largest posterior probability was found. Those samples that presented a probability lower than 60% remained unclassified. These classes can be further used to define a classification procedure for the dynamic images.

## 3 Results

Sequences used for each type of disorder were successful, as it was possible to identify the internal problem in each case (Fig. 2).

### *3.1 Histogram parameter analysis*

For **watercore disorder**, mean gray level, variance, skewness and kurtosis were obtained from the histogram of the dynamic images and an ANOVA was performed on both masks, using an interaction of both, the three repetitions and the visual classification assigned by the three experts (Fig 3). Results showed robustness on the three repetitions in all the studied parameters and significant differences were found in class 4 for mean gray level (F=15.4), variance (F=11.7) and skewness (F=11.9). No significant differences were found for kurtosis. Only damage level 4 can be segregated used features extracted from the histograms. Both masks provided similar results.

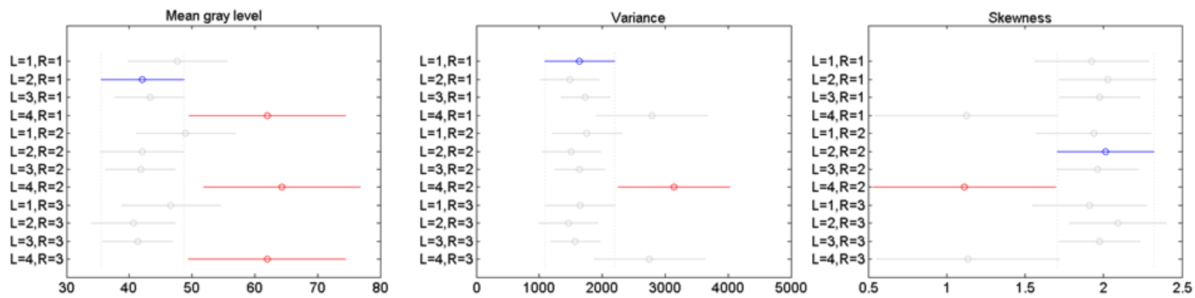


**Fig. 3** ANOVA on mean gray level, variance and skewness respectively. L corresponds to the class assigned by the three experts (1, 2, 3 or 4) and R to the repetition.

For Braeburn disorder, this methodology was followed, as it was seen that the protocol was successful. In this case no significant differences were found, as only 12 fruits were imaged and the dispersion of the means was very high (data not shown).

### *3.2 Results on PLS*

When using the normalized histogram of the apple tissue in static images for the generation of a PLS model to estimate the damage percentage found with the interactive 3D analysis, the correlation coefficient found is 0.92.

For the dynamic images results on corresponding PLS models were very similar for both mask procedures. For mask 1, the correlation coefficient values were, respectively for the three dynamic repetitions, 0.72, 0.68, 0.69. For mask 2, they were 0.74, 0.70, 0.55.

When plotting the observed against the predicted individuals (Fig 4) a lineal trend is found. In general, individuals with a high amount of damage are well classified by the model. Several samples with no damage or a low percentage of damage show a particular error trend that needs further analysis.

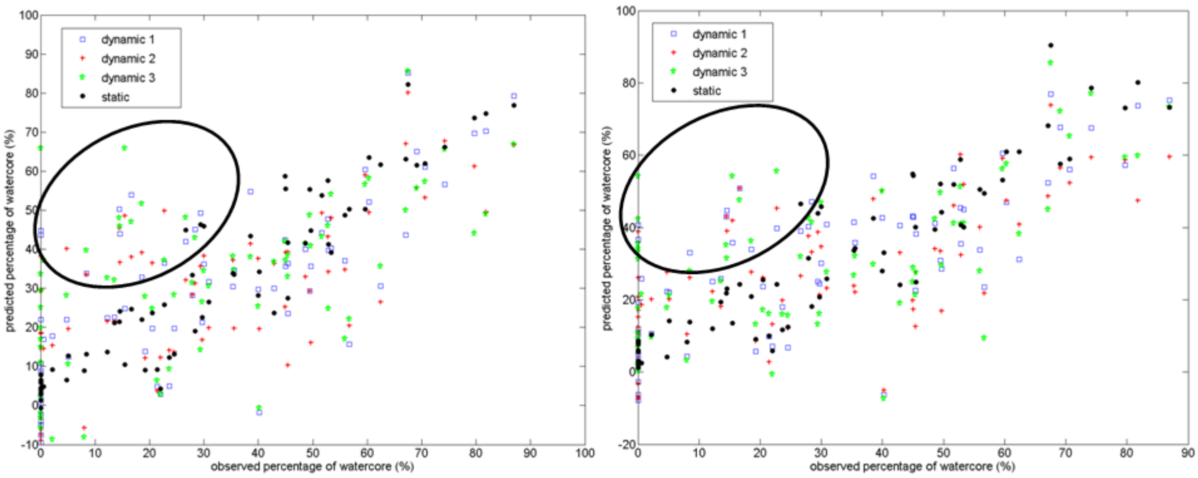


**Fig. 4** Observed percentage of watercore, computed with an interactive segmentation, against predicted percentage of watercore, performed with a PLS model. Some observed individuals with low damage percentage are classified with higher damage percentage. Strongly affected individuals present a good classification by the model.

### *3.3 Error analysis and Bayesian classification*

From the error analysis of the three PLS models (one for each replicate) it can be said that the estimation error does not depend on repetition which can fluctuate in the position of the sample in the magnet. Nevertheless, it seems it might be related to a specific Euler number (around -400, data not shown) which correspond to apple with peripheral and moderate damage (not all slices are equally affected). It has to be taken into account that the dynamic image is without slice selection for the watercore sequence and thus such peripheral defects may generate an over-detection artifact in the dynamic image.

The reliability of experts classification can be confirmed by the confusion matrix from the Bayesian classification (Table 1), 54 individuals presented a good visual classification, which is the 76% and 10 were misclassified (14%). 7 samples (~ 10%) were no classified by the model, as their class belonging was less than 60%. Therefore expert classification can be used to generate PLS classification models without the need for making 3D interacting segmentation in static images and thus so will be done for upcoming validation trials.

**Table 1** Observed individuals against Bayesian classification

| | | **Observed** | | | |
|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** |
| **Bayesian** | **1** | 13 | 0 | 0 | 0 |
| | **2** | 1 | 16 | 6 | 0 |
| | **3** | 0 | 1 | 18 | 0 |
| | **4** | 0 | 0 | 2 | 7 |

## 4 Discussion

### *4.1 Sequence selection*

According to previous studies on on-line MRI (Hernandez-Sanchez et al., 2004, 2005, 2006), in order to minimize motion artifacts, fast imaging sequences were used. FLASH sequence maximizes tissues differences, as it is weighed in T2*. This kind of sequence was successful for seeds detection in mandarins, due to the high contrast that exists between the pulp of the citrics (with high amount of water) and the seeds (that will be seen darker because of the absence of water). This sequence provided also good results for one of the disorders studied in this work: watercore. The damage was clearly seen as there is a high contrast between tissues (due to watercore disorder is characterized by free water in the tissues, which will be seen brighter than the rest of the fruit). Nevertheless, it did not provide good results in the study for internal breakdown, as the pulp of the fruit is not visible when using gradient echoes T2*weighted sequences. For such purpose, a spin echo T2 weighed sequence was used, UFLARE.

### *4.2 Image analysis*

Automated segmentation procedures for dynamic images have shown to be complex since there is a need for at least 4 or 5 steps though the result is promising and can be directly implemented on-line provided the computing resources are not limited.

Features extracted from histograms are not enough to segregate between four damage levels, only assessing the highest level of affection. Instead, the use of PLS models based on the whole histogram do allow such performance.

## 5 Conclusions

Each disorder among which watercore and internal breakdown are extreme cases requires the definition and adjustment of particular sequences which in any case require the correction of motion artifacts. In those cases where the image contrast is poor more restrictive number and variety of sequences is available.

A complete procedure for watercore has been defined, from sequence definition, motion correction, automated image segmentation and feature extraction. It has been validated against quantitative and qualitative values from expert classification. Damage estimation performance is maximum for static images (r=0.92) and acceptable for dynamic images (r= 0.72; 0.68; 0.69 respectively for each repetition of mask 1 and r= 0.74; 0.70; 0.55 respectively for each repetition of mask 2). No performance differences are found between image replicates which confirm the MRI dynamic procedure to be robust and prepared of industrial evaluation.

## Acknowledgements

We would like to thank to the EU project InsideFood (FP7-226783) for financial support. The opinions expressed in this document do by no means reflect their official opinion or that of its representatives.

## References

Barreiro, P., Zheng, C., Sun, D-W., Hernandez-Sanchez, N., Perez-Sanchez, JM., PerezSanchez, JM, Ruiz-Cabello, J., 2008. Non-destructive seed detection in mandarins : comparison of automatic threshold methods in FLASH and COMSPIRA MRIs. Postharvest Biol. Technol. 47, 189-198.

Blasco, J., 2012. Magnetic resonance imaging detects seed in mandarins. SPIE, 10.1117/2.1201201.004049.

Clark, C., MacFall, J., Bieleski, R., 1998. Loss of watercore from 'Fuji' apple observed by magnetic resonance imaging. Scientia Hort. 73(4), 213-227.

Hernandez-Sanchez, N., Barreiro, P., Ruiz-Altisent, M., Ruiz-Cabello, J., Fernandez-Valle, ME., 2004. Detection of freeze injury in oranges by Magnetic Resonance Imagign of moving samples. Appl. Magn. Res. 26, 431-445.

Hernandez-Sanchez, N., Barreiro, P., Ruiz-Altisent, M., Ruiz-Cabello, J., Fernandez-Valle, ME., 2005. Detection of sedes in citrus using Magnetic Resonance Imaging under motion conditions and improvement with motion correction. Concepts in Magn. Res. Part B: Magn. Reson. Eng. 26B, 81-92.

Hernandez-Sanchez, N., 2006. Development of on-line NMR applications for the evaluation of fruit internal quality. Thesis.

Hernandez-Sanchez, N., Barreiro-Elorza, P., Ruiz-Cabello, J., 2009. Optical monitoring of fresh and processed agricultural crops. Chapter tittle: NMR for internal quality evaluation in horticultural products. Ed. CRC PRESS 423-468.

McCarthy, M.F., 1994. Magnetic Resonance Imaging in Foods, 1st ed., 9.3. New York: Chapman and Hall.

Melado-Herreros, A., Muñoz-García, M-A., Blanco, A., Val, J., Fernandez-Valle, M-E., Barreiro, P., 2012. Relationship between solar radiation on watercore on Apple fruit assessed with MRI. Poster. International Conference of Agricultural Engineering (CIGR-AgEng2012).

Min Kim, S., Milczarek, R., McCarthy, M., 2008. Fast detection of seeds and freeze damage of mandarins using magnetic resonance imaging. Modern Physics Letters B 22 (11), 941-946.